Title

Happy faces selectively increase the excitability of cortical neurons innervating frowning muscles of the mouth.

Abstract

Although facial muscles are heavily involved in emotional expressions, there is still a lack of evidence about the role of face primary motor cortex (face M1) in the processing of facial recognition and expression. This work investigated the effects of the passive viewing of different facial expressions on face M1 and compared data with those obtained from the hand M1. Thirty healthy subjects were randomly assigned to two groups undergoing transcranial magnetic stimulation (TMS) of face or hand M1. In both groups, short-latency intracortical inhibition (SICI) and intracortical facilitation (ICF) were probed in the depressor anguli oris (DAO) and first dorsal interroseus (FDI) muscles 300 ms after presentation of a picture of a face that expressed either happy, sad or neutral emotions. Statistical analysis of SICI showed a non-significant effect of muscle ($F_{1,28}$= 1.903, p= 0.179) but a significant effect of emotion ($F_{2,56}$=6.860, p=0.004) and a significant muscle X emotion interaction ($F_{2,56}$=5.072, p=0.015). Post hoc analysis showed that there was a significant reduction of SICI in the DAO muscle after presentation of a face with a happy expression compared with a neutral face (p<0.001). In the FDI, a significant difference was observed between neutral and sad expressions (p=0.010) No clear differences in ICF were detected. The different responses of face and hand muscles to emotional stimuli may be due to their functional roles in emotional expression versus protection of the body.

Key words: face primary motor cortex, hand primary motor cortex; TMS; emotional motor control; volitional motor control; face expressions.

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Introduction

In humans, the ability to recognize and react to facial expressions rapidly is crucial for survival and social communication (Blair 2003, 2004). Neurobiological models propose that the recognition of face expressions involves the coordinated participation of multiple cortical areas such as the inferior occipital gyrus and the superior temporal sulcus (Haxby et al. 2000; Rossion et al. 2003; Calder and Young 2005; Engell and Haxby, 2007). The recognition of facial expression is automatic and fast; indeed, it takes only 300 ms to process the emotional content of a picture (Smith and Smith 2019). Over the same period, information is also sent to the motor and premotor areas (Cuthbert et al. 2000). Previous work has shown that recognition of facial expression depends not only on activity in the right occipital face area, but also involves activity in right somatosensory cortex, confirming the idea that facial expression recognition is not solely a visual task (Pitcher et al. 2008). In addition to visual and contextual routes to emotion recognition, a new theoretical model called "sensorimotor stimulation" has recently proposed that people subliminally recreate in their own motor system the commands involved in the facial expression being viewed (Wood et al. 2016a, b). This subthreshold activity, in theory, triggers partial, often unconscious, activity in other neural systems involved in experiencing the corresponding emotion, and from which the viewer implicitly infers the expresser's internal state (Wood et al. 2016a, b; Gallese 2005). However, activation of the somatosensory stimulation system depends on several factors, such as the difficulty of the task as well as individual and behavioral features (Wood et al. 2016a).

Several studies in healthy volunteers have demonstrated that viewing facial expressions triggers a cascade of central and peripheral physiological processes associated with action preparation (Dalgleish 2004; Vuilleumier and Pourtois 2007) involving anatomo-functional connections between the limbic associative cortex and the premotor/motor areas, via the cingulate and prefrontal cortical regions (Vuilleumier and Pourtois 2007). Interestingly, several human studies using transcranial magnetic stimulation (TMS) of the hand primary motor cortex (M1) demonstrated an increase in corticospinal tract excitability in response to emotional stimuli relevant for action of the whole body such as pleasant or unpleasant scenes (Oliveri et al. 2003; Baumgartner et al. 2007; Hajcak et al. 2007; Schutter et al. 2008; Coombes et al. 2009; Hortensius et al. 2016). Moreover, previous studies demonstrated that the pre-SMA plays a role in facial happiness recognition (Rochas et al. 2013). However, only one previous study employed emotional facial expressions such as fear and found an increase of M1 excitability in the abductor pollicis brevis muscle (Shutter et al. 2008).

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Given their role in emotional expression, it seems odd that there have been few studies (but see Muri 2016) of the effect of viewing facial emotions on facial muscles themselves. Indeed, facial expressions are part of stereotyped physiological responses to peculiar affective states involving both the autonomic and somatic systems and are controlled by the so-called "emotional motor system" (Holstege 1992; Holstege et al. 1996), which appears to be quite separate from the corticobulbar system that mediates volitional movement. Thus, patients have been described in whom focal lesions to a variety of areas including the contralateral thalamus, anterior striato-capsular region, medial part of frontal lobes (Bogousslavsky et al. 1988; Trosch et al. 1990; Ross and Mathiesen 1998; Hopf et al. 1992) and the ipsilateral pons and medulla (Khurana et al. 2002; Cerrato et al. 2003) can have isolated emotional facial palsy in the absence of effects on voluntary contraction of the same muscles. The same division is seen in the much more frequent condition of voluntary facial palsy, with sparing of emotional movements, that can occur after brainstem lesions (Trepel et al. 1996; Bouras et al. 2007).

Previous work using TMS has shown that it is possible to study motor control of facial muscles (Cruccu et al. 1990; Paradiso et al. 2005; Cattaneo and Pavesi 2014) in a variety of muscles such as lip depressors (Meyer et al. 1994), muscles active in pursing of lips (Triggs et al. 2005), the buccinator muscle (Urban et al. 1997), and the depressor anguli oris (DAO) (Pilurzi et al. 2013). Pilurzi et al (2013) also showed that it was possible to evaluate short-latency intracortical inhibition (SICI) and facilitation (ICF) in both the ipsilateral and the contralateral motor representations of the DAO (Pilurzi et al. 2013).

In the present study we have therefore used these methods to examine the effect of viewing faces expressing different emotions on the excitability of the face area of human motor cortex. We compared the results with the effect of the same stimuli on the excitability of the motor cortex hand area, since hand muscles may also be involved in expressing different emotions.

Methods

Participants

Thirty healthy subjects (21 females and 9 males; mean age 26.47± 5.09 years), all right-handed according to the Oldfield inventory scale (Oldifield 1971), participated in the study. An informed written consent was obtained from all subjects and the experimental procedure was approved by the

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local ethical committee (Bioethics Committee of ASL. n. 1 – Sassari, ID 2075/CE/2014) and conducted in accordance with the Helsinki Declaration. None of the participants had history and/or current signs/symptoms of neurological and/or psychiatric diseases. Recordings were carried out in a quiet room while subjects were seating in a comfortable chair and were asked to stay relaxed but alert during the experiment.

EMG

EMG was recorded from right DAO and first dorsal interosseous (FDI) using a 9 mm diameter Ag-AgCl surface electrodes. For the DAO recording, the active electrode was placed at the midpoint between the angle of the mouth and the lower border of the mandible, the reference electrode over the mandible border, 1 cm below the active electrode, and the ground electrode over the right part of the forehead. For the FDI EMG recordings, the active electrode was placed over the muscle belly, the reference electrode at the second metacarpo-phalangeal joint and the ground electrode over the forearm. EMG signals were recorded (D360 amplifier, Digitimer Ltd, Welwyn Garden City, UK), amplified (x1000), filtered (bandpass 3-3000 Hz) and sampled at 5 KHz using a 1401 power analog-to-digital converter and Signal 6 software (Cambridge Electronic Design, Cambridge, UK). The DAO and the FDI muscles were chosen as models for the face and hand muscles, respectively, since the protocols used in the present study have been already standardized (Pilurzi et al. 2013; Rossini et al. 2015).

TMS

TMS was performed using a 70 mm figure-of-eight shaped coil connected to a Magstim 200 stimulator stimulators through a Bistim module (Magstim Co., Whitland, and Dyfed, UK). The optimal stimulation site for the DAO and FDI, defined as the cortical spot where larger motor evoked potentials (MEP) were obtained, was carefully searched and then marked with a soft tip pen over the scalp, to maintain the same coil position throughout the experiment. For the DAO, the handle of the coil pointed posteriorly and laterally, at approximately 30-45 deg to the interhemispheric line (Pilurzi et al. 2013, 2020; Ginatempo et al. 2019), while for FDI it was pointing backwards and laterally at 45° away from the midline (Rossini et al. 2015). The resting motor threshold (RMT) was defined as the lowest TMS intensity that elicited, in the relaxed muscle, MEPs of at least 0.05 mV in at least 5 out of 10 consecutive trials and was expressed in percentage of the maximum stimulator output (Rossini et al. 2015). Paired-pulse TMS protocol was delivered with the same coil, the stimuli consisted of a subthreshold conditioning stimulus (CS) preceding a

suprathreshold test stimulus (TS) by an interstimulus interval (ISI) of 3 ms for short-latency intracortical inhibition (SICI) and 10 ms for intracortical facilitation (ICF). In both cases the CS intensity was set at 80% RMT and the TS intensity at 120% RMT. SICI and ICF were expressed as the ratio of MEP amplitude evoked by the conditioned to the unconditioned MEP.

Facial emotional expressions stimuli (FES)

The visual stimuli consisted of photographs of ten actors taken from the Karolinska directed emotional faces set (Lundqvist et al. 1998). Each actor (10 in total, 5 women) displayed a neutral, sad or happy facial expression for a total of 30 visual stimuli. All stimuli were projected on a 17" CRT monitor, with a 1280x1024 resolution and a 70 Hz refresh rate, by using PsychToolbox software (Brainard 1997), running in MATLAB environment (Version 2015b, MathWorks, Inc., Natick, MA, United States). The present experimental procedure employed the same protocol used by Schutter and colleagues (2008). Previous ERP studies of Smith and Smith (2019) suggested that decoding of face identity and expression is maximal in a 90–170 ms time-period post-stimulus whereas Carlsen et al (2011, 2013) give a slightly wider interval of 80 – 200ms for the read-out of face exemplar information from whole brain. Since Adolphs (2002) suggest that additional time is required to develop conceptual knowledge of the emotion signaled by the face (>300 ms), we chose a time interval of 300 ms between the onset of the visual stimulus and the TS. The inter-trial interval varied randomly between 4800 and 5200 ms.

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Experimental design

The study comprised a main experiment (experiment 1) and one control experiment (experiment 2).

Experiment 1: Influence exerted by facial emotional expressions stimuli (FES) on the M1 innervating facial and hand muscles.

Experiment 1 was planned to investigate a possible effect of the passive viewing of emotional stimuli on hand M1 and face M1, recording the TMS-induced MEPs in the FDI and DAO muscles, respectively. In order to reduce the number of the stimuli delivered to each subject, all subjects were divided up into two groups: paired-pulse in DAO (5 males and 10 females, 26.67±4.47 years old) and paired-pulse in FDI (4 males and 11 females, 26.87±2.77 years old). Both SICI and ICF were tested in DAO and FDI M1s after 300 ms from the delivery of FES (neutral, sad and happy faces).

Twenty unconditioned and 20 conditioned MEPs for each ISI and condition were recorded in randomized order.

Experiment 2. Arousal rating of FES

All Participants were asked to rate each picture on a visual analog scale (VAS) based on their affective response to the FES used in experiment 1. Participants were asked how they felt after seeing each facial expression and to rate the level of arousal on the VAS indicating a number from 1 (no visceral response) to 9 (very strong visceral response). The mean of the rating for each emotional condition was calculated and used for the statistical analysis.

Statistical analysis

Raw amplitude and amplitude ratio of conditioned to unconditioned MEPs, were used as variables.

Statistical analysis was performed with SPSS 20 software (SPSS Inc, Chicago, IL, USA). Mixed and repeated measures (RM) analysis of variance (ANOVA) and planned post hoc t-tests with Bonferroni correction for multiple comparison were used. Compound symmetry was evaluated with the Mauchly's test and the Greenhouse-Geisser correction was used when required. Significance was set for p values < 0.05. Value are expressed as mean ± standard deviation.

Experiment 1: A preliminary RM- ANOVA on raw TS MEP amplitude was performed separately for each MUSCLE GROUP (DAO and FDI) with EMOTION (happy, sad and neutral) as within-subjects factor. In case of no significant effect of emotion on MEP amplitude was detected, a two-way mixed-ANOVA separately for SICI and ICF, was performed using amplitude ratio as variable with EMOTION (happy, sad and neutral) as within-subjects factor and MUSCLE GROUP (FDI and DAO) as between-subjects factor.

Experiment 2: a two-way RM-ANOVA on the rating was performed with EMOTION (happy, sad and neutral) as within-subjects factor and MUSCLE GROUP (FDI and DAO) as between-subjects factor. When a significant effect was detected in the Experiment 1, a correlation analysis was performed between the rating attributed to each FES and the amplitude of MEP for TS, SICI and ICF ratio, using Spearman's correlation coefficient.

Results

Experiment 1: Influence exerted by facial emotional expressions stimuli (FES) on the M1 innervating facial and hand muscles.

The RMT in the DAO face M1 was 52.3 ±14.5% maximum stimulator output while in the FDI M1 was 41.6±8.3% of the maximum stimulator output. A preliminary analysis of the test MEP amplitude to a single TMS pulse alone showed no significant effect of EMOTION for either MUSCLE GROUP (DAO: $F_{1,28}$ = 2.948, p=0.070, effect size (ES)=0.525; FDI: $F_{1,28}$ = 0.053 p=0.944, ES=0.057) (Figure 1). Given the lack of effect on the test MEP amplitude, we then proceeded to analyse effects of the visual stimuli on the SICI and ICF ratios.

A mixed ANOVA on SICI ratio showed a non-significant effect of MUSCLE GROUP ($F_{1,28}$= 1.903, p= 0.179, ES=0.266) but a significant effect of EMOTION ($F_{2,56}$= 6.860, p= 0.004, ES=0.859) and an interaction between the two factors ($F_{2,56}$= 5.072, p= 0.015, ES=0.735). Specifically, the main effect of emotion was driven by viewing sad and happy expressions compared with neutral faces (neutral *versus* sad: T_{29}= 3.1964; p=0.009; neutral *versus* happy T_{29}=3.6514; p= 0.001) in both muscles. In particular, post-hoc analysis of the interaction between the two factors detected a significant reduction of SICI in the DAO muscle when viewing happy expressions compared with neutral expressions (p<0.001). In the FDI, a significant difference was observed between neutral and sad expressions (p= 0.010) (Figure 2). Moreover, SICI in DAO was reduced more than SICI in FDI viewing happy expressions (FDI *versus* DAO: T_{29}= 2.390; p= 0.026).

The mixed ANOVA on ICF ratio showed a non-significant effect of MUSCLE GROUP ($F_{1,28}$= 0.001, p= 0.972, ES=0.050), EMOTION ($F_{2,56}$= 1.556, p= 0.218, ES=0.318) and no interaction between the two factors ($F_{2,56}$= 1.673, p= 0.198, ES=0.330) (Figure 3).

Experiment 2. Arousal ratings of FES

A two–way RM-ANOVA detected a significant effect of EMOTION ($F_{2,56}$= 36.570; p<0.001; ES=1.00) but a non-significant effect of MUSCLE GROUP ($F_{1,28}$= 0.001; p=0.980; ES=0.050) or interaction among the factors ($F_{2,56}$= 0.116; p=0.839; ES=0.065). The post-Hoc analysis showed that the lowest rating was observed following neutral stimuli, although all the FES were significantly different from each other (neutral: 1.55±0.34; sad: 2.85±0.26; happy: 4.51±0.29; all p<0.05). Spearman analysis failed to detect any significant correlation between rating values and SICI ratio (all p>0.05).

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Discussion

The present study demonstrated, for the first time, a significant relationship between passive viewing of facial expressions and excitability of inhibitory and excitatory connections in face M1. Moreover, there was a clear difference between the effects on face and hand M1 with facial expressions of happiness affecting excitability of face M1 whereas sadness influenced hand M1.

Our main finding of reduced SICI in the FDI following vision of sad faces, compared to neutral and happy faces, is in line with previous work (Hajcak et al. 2007; Schutter et al. 2008; Coombes et al. 2009) and favors the hypothesis that activation of the fight/flight response, induced by unpleasant stimuli, is responsible for increased excitability (i.e. reduced inhibition) of the hand M1 (Hajcak et al. 2007; Schutter et al. 2008). In addition, neuroimaging studies demonstrated that during passive viewing of unpleasant stimuli such as sad, fear and disgusting stimuli, brain structures involved in detection and reaction to danger are constantly active (Morris et al. 1999) to prepare the organism for a rapid action crucial for survival (Anderson and Phelps 2001). According to this logic, the link between positive emotions and hand motor behavior would appear to be less relevant since there is no associated value in evolutionary terms (Baumgartner et al. 2007).

In contrast with the study from Schutter et al. (2008), we did not observe any effect on the test MEP. Their study used the abductor pollicis brevis as the target muscle and the same hand was used to press a button to identify the facial expression (Schutter at al. 2008) while in our experiments, the subjects had to view the faces passively. The lack of any specific task may account for the absence of any effect on the FDI MEP, since it has been reported that the processing of faces depends on the task required of participants when they view them (Smith and Smith 2019).

In contrast to the results in FDI, the excitability of face M1 projecting to the DAO muscle was unaffected by sad faces. It is possible that aversive stimuli elicit more bodily than facial responses, since changes in the program of action may be more important than changes in facial expressions.

Anatomical considerations

Face movements are directly linked to emotions and play a major role in non-verbal communication and in social behavior (Müri 2016). The anatomy of brain areas that send inputs to the facial

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nucleus was explored in some detail by Morecraft et al. (2001) (see also reviews by Cattaneo and Pavesi 2014; Muri 2016). Five cortical areas project onto the facial nucleus: face M1, the ventral lateral premotor cortex (LPMCv), the supplementary motor area (SMA or M2), the rostral cingulate motor cortex (M3) and the caudal area of the anterior mid-cingulate (M4). Volitional inputs to lower facial muscles are thought to come mainly from M1 and LPMCv whereas emotional inputs come from M3. Within the volitional system, the LPMCv receives connections from cingulate and parietal territories mostly related to face and mouth movements as well as with the anterior and mid-dorsal part of the insula, whose electrical stimulation is known to evoke disgust-related behaviors (Caruana et al. 2011) and affiliative facial expressions (Jezzini et al. 2012). The LPMCv also receives input from the areas of prefrontal cortex involved in visual coding of biological motion and facial expressions (Petrides and Pandya 2002; Gerbella et al. 2010, 2011; Ferrari et al. 2017), which are known to contain neurons responding to visual stimuli of faces and of facial communicative gestures (Ó Scalaidhe et al. 1997, 1999; Ferrari et al. 2017). Thus, the volitional system will also be affected by visual inputs. We speculate that the change in SICI within M1 produced by viewing happy faces may utilize this pathway and that the influence on LPMCv is conveyed to face M1 by the known connections between these structures.

The anatomy of visual influences on hand movement is quite different. The medial PMCv (MPMCv) projects to the hand area of M1 and receives visual inputs mainly from the anterior inferior-parietal area (Rozzi et al. 2006; Borra et al. 2008; Bonini et al. 2010, Ferrari et al. 2017).

The influence of happy faces on the DAO, which is a muscle usually associated with sadness, was unexpected. Indeed, several studies demonstrated that happy faces produce EMG activity in the "smiling" muscles, such as zygomatici and orbicularis oculi, while negative expressions produce activity in the corrugator supercilia muscle (Müri 2016). However, these muscles are not easy to study with TMS protocols: perioral muscles have a wider cortical representation and a lower threshold to TMS than other muscles of the face (Cattaneo and Pavesi 2014). Moreover, all the TMS protocols used in the present work have been already standardized in the DAO (Pilurzi et al. 2013), which is the reason why this muscle was chosen as a model, although it has seldom been investigated in the face expression studies. We speculate that the influence exerted by viewing happy faces on face M1, via the LPMCv, is not muscle-specific and may well involve facial muscles other than the DAO, which were not investigated in the present study.

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Conclusions

The present findings provide evidence that the M1 face area innervating the frowning muscles of the mouth is selectively modulated when viewing faces expressing happiness. In contrast, viewing faces expressing sadness selectively modulates hand M1. The different responses of face and hand muscles may relate to the different physiological role of these muscles: the former mainly involved in social communication, the latter in the protection of the body from aversive stimuli. This work may pave the way for future studies aimed at clarifying the physiopathology of facial muscle disorders, in which not only the voluntary but also the emotional motor systems are involved.

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Conflict of interest

The authors declare no conflicts of interest.

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583

584 **Figure Legends**

585 **Figure 1: Influence exerted by facial emotional expressions stimuli (FES) on MEP amplitude**
586 **for both FDI and DAO muscle.**

587 The graph shows amplitude of unconditioned MEP, for both FDI and DAO muscles, following
588 visual stimuli of happy, sad and neutral face expressions. No clear effect of the different visual
589 stimuli was detected for each muscle group. The graph represents means + SEM.

590

591 **Figure 2: Influence exerted by facial emotional expressions stimuli (FES) on the SICI protocol**
592 **for both FDI and DAO muscle.** The graph shows MEP amplitude expressed as a percentage of
593 unconditioned MEP, for both FDI and DAO muscle during SICI protocol following happy, sad and
594 neutral visual stimuli. SICI protocol showed a significant reduction following happy in DAO
595 muscle while in FDI following sad conditions. The graph represents means + SEM. *$p < 0.05$.

596

597 **Figure 3: Influence exerted by facial emotional expressions stimuli (FES) on the ICF protocol**
598 **for both FDI and DAO muscle.** The graph shows MEP amplitude expressed as a percentage of
599 unconditioned MEP, for both FDI and DAO muscle during ICF protocol following happy, sad and
600 neutral visual stimuli. No clear modulation was detected for both muscle. The graph represents
601 means + SEM. *$p < 0.05$.

Fig. 5

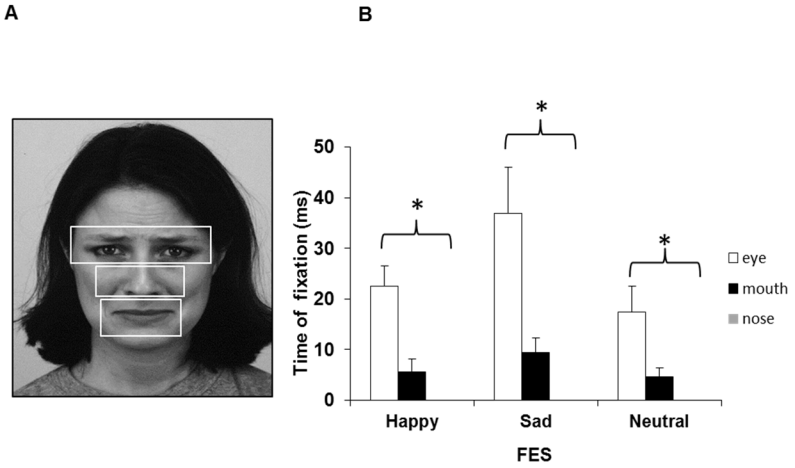
A
B
Time of fixation (ms)
50
40
30
20
10
0
*
*
*
Happy
Sad
Neutral
FES
eye
mouth
nose

Fig.4

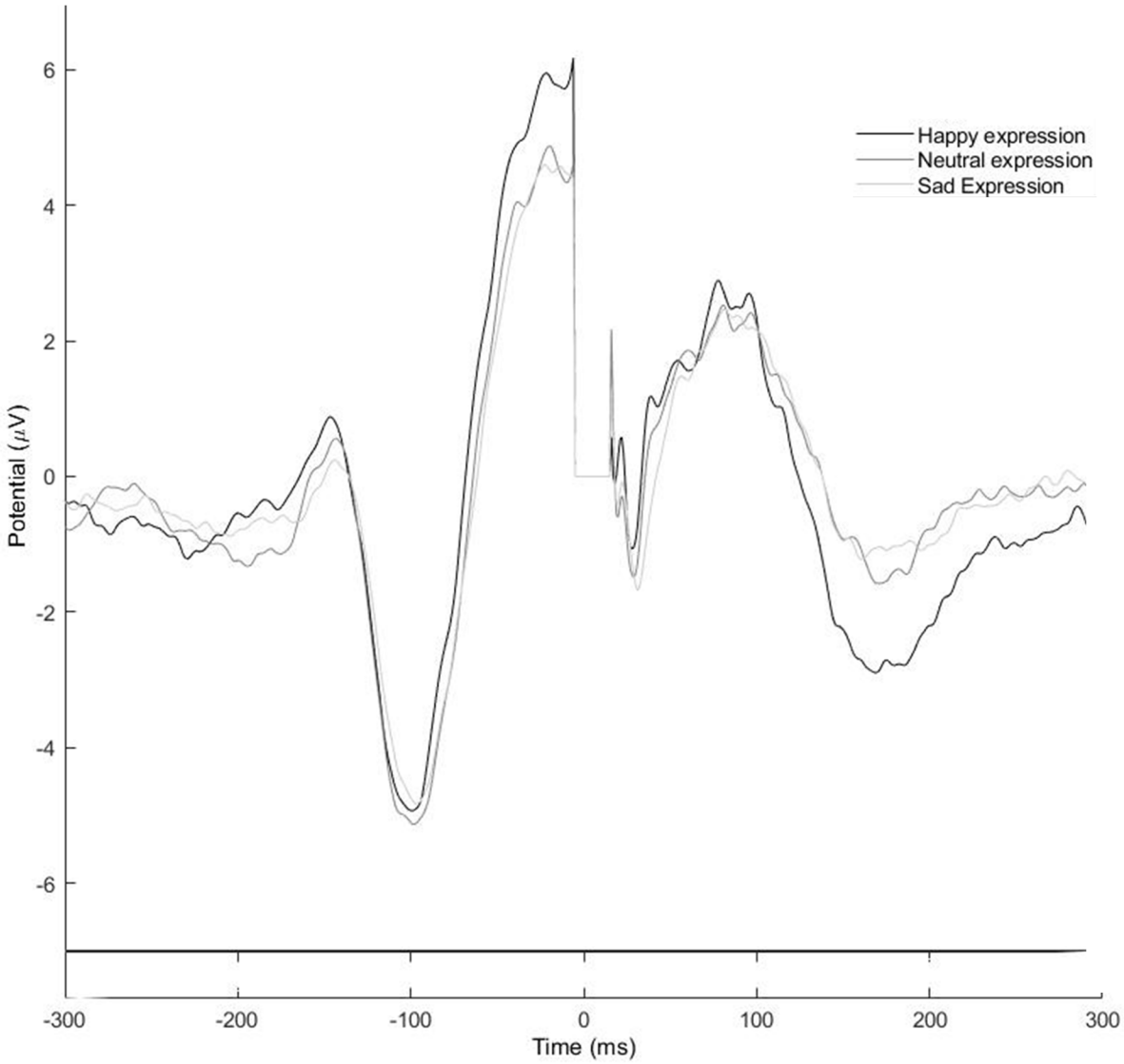
Happy expression
Neutral expression
Sad Expression
Potential (μV)
6
4
2
0
-2
-4
-6
-300
-200
-100
0
100
200
300
Time (ms)

Fig.3

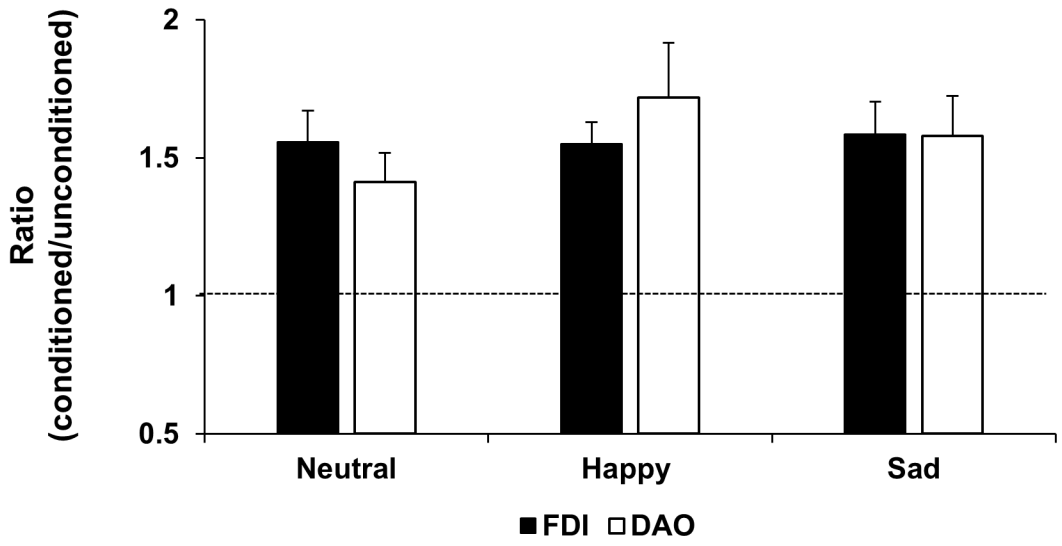
Ratio
(conditioned/unconditioned)
2
1.5
1
0.5
Neutral
Happy
Sad
FDI
DAO

Fig.2

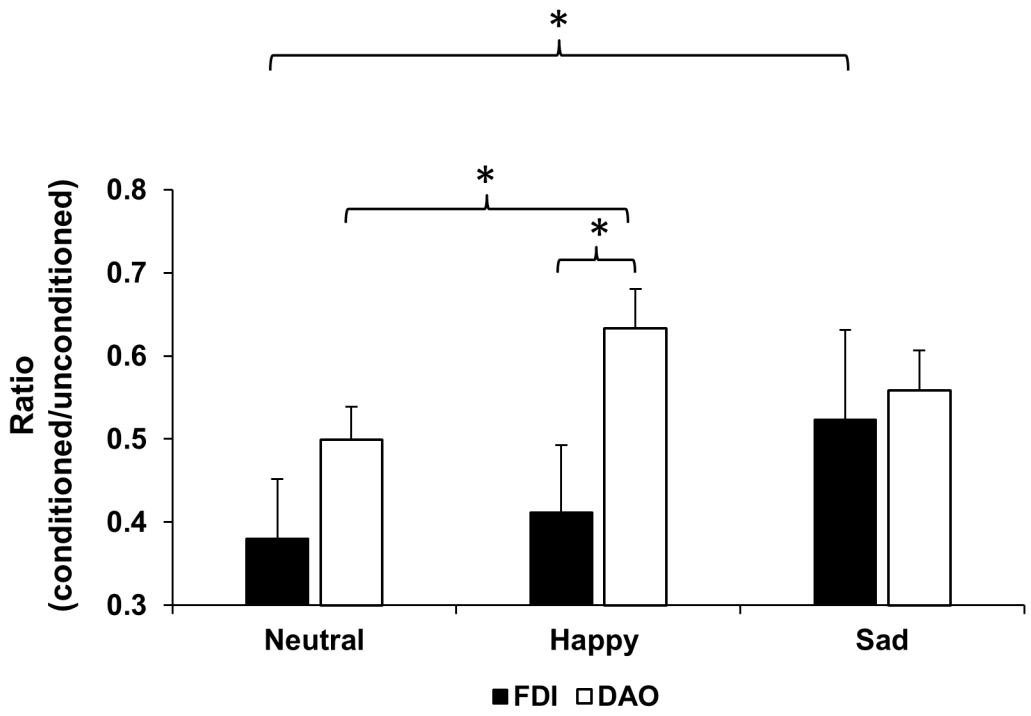
*
*
*
Ratio
(conditioned/unconditioned)
0.8
0.7
0.6
0.5
0.4
0.3
Neutral
Happy
Sad
FDI
DAO

Fig.1